

Determination of Molecular Weight

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An initial study, in which 23 collaborators participated, was conducted on the determination of molecular weight by the isothermal distillation (vapor pressure osmometry) method. Each used his own method and apparatus to analyze four samples in duplicate; the molecular weight ranged from 123 to 891. Results show that the chief problem is the choice of the correct solvent and reference standard and that the use of bare thermistors is questionable.

The two preliminary studies on micro methods for the determination of molecular weight (1, 2) indicated that the isothermal distillation method using thermistor probes (vapor pressure osmometer) was the preferred method. In 1959, only two collaborators, using homemade instruments, participated in the study; consequently, further work was delayed until more laboratories were equipped to perform the analyses. This year, 25 of the 60 collaborators contacted had the appropriate apparatus and wished to participate in the study; 23 reported results. Many more laboratories not on our collaborator list are now using this method for determining molecular weight.

Collaborators were asked to analyze 4 samples in duplicate, using the apparatus and techniques they would normally use in their laboratory, and to return, with their results, details of their method including solvent and reference standard used, sample weights, solvent weights or volumes, temperature, equilibration time, techniques of measurements and apparatus details. The following six samples were used in the study: (1) benzylisothiurea hydrochloride; (2) sulfanilamide; (3) benzoic acid; (4) *N*(*n*-

octadecyl)stearamide; (5) nicotinic acid; (6) tristearin. Samples 1, 2, 3, and 4 were sent to approximately half the collaborators and samples 1, 2, 5, and 6 to the other half.

Results and Discussion

Sample 1, benzylisothiurea hydrochloride, was included among the samples to ascertain how an ionizable organic compound would be analyzed by the various collaborators. Sample 2, sulfanilamide, a compound presenting no particular problems, was also sent to all collaborators. Samples 3 and 5, benzoic and nicotinic acids, were chosen because they contain a carboxyl group which tends to cause dimerization unless strongly polar solvents are used. Samples 4 and 6, *N*(*n*-octadecyl)stearamide and tristearin, were selected because of their relatively high molecular weights. Unfortunately, sample 4 was not soluble in most of the commonly used solvents. Collaborators were instructed to omit this sample if they could not find a suitable solvent.

The results of the study, shown in Table 1, include duplicate and average values for each sample analyzed by each collaborator and laboratory standard deviations, σ , calculated from the difference between duplicates. Data from samples 4 and 6 were not used in the calculation of σ because of their relatively high molecular weights and because of solubility problems with sample 4.

Interlaboratory standard deviations and coefficients of variation are shown at the bottom of Table 1 for all samples except the first. Statistical data on sample 1 would be meaningless because most collaborators did not use an ionizable reference standard. Data from collaborators 6, 19, and 25 were omitted in calculating the standard deviation for sample 3, since the molecular weight values were high because of the solvents used. The data for benzylisothiurea hydrochloride

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(sample 1) range from 104 to 206, depending on the solvent and reference standard used. This sample contains an ionizable chloride; therefore, those collaborators who did not use a polar solvent with an ionizable reference compound obtained low results, whereas those who used an appropriate solvent and standard obtained good results ranging from 201 to 206 vs. a theoretical value of 202.7. Table 2 shows the solvent and reference compound used by each collaborator for each sample analyzed.

The values reported for sample 2, sulfanilamide, are generally good. The mean value for the 23 collaborators is 172.3 vs. 172.2, but standard deviation and coefficient of variation (c.v.) are 10 and 5.8, respectively. Comparison of the individual collaborator's values with the information in Table 3 indicates that the use of bare

thermistors might be related to the more divergent values reported. The statistical values calculated for those who used coated thermistors were $\bar{X} = 170.3$, $\sigma = 7.0$, and c.v. = 4.1; for those who used bare thermistors, $\bar{X} = 177.7$, $\sigma = 15$, and c.v. = 8.4. Eliminating the one low value of 146, the data for coated thermistors becomes $\bar{X} = 171.7$, $\sigma = 3.1$, and c.v. = 1.8. These values, although not conclusive, raise a question as to the advisability of using bare thermistors.

Benzoic acid (sample 3) tended to dimerize unless a highly polar solvent such as water or alcohol was used. Most collaborators used such a solvent and obtained good results. Those who used a nonpolar solvent obtained about twice the theoretical molecular weight.

Because sample 4, *N*(*n*-octadecyl)stearamide, was sparingly soluble in the commonly

Table 1. Molecular weight values obtained in collaborative study

Coll. No.	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		σ
	M.W. 202.7		M.W. 172.2		M.W. 122.1		M.W. 536.0		M.W. 123.1		M.W. 891.5		
	Av.		Av.		Av.		Av.		Av.		Av.		
0	204		172		123		538		125		912		3.0
	197	201	172	172	121	122	538	538	121	123	870	891	
0			170		123								—
			176	173	125	124							
1	188		165		124		494						7.9
	203	196	169	167	126	125	510	502					
2	112		175		120								2.5
	109	110	170	173	121	121							
3	202		180		128								21.9
	206	204	232	206	142	135							
6	128		172		252		556						7.2
	122	125	186	179	243	248	558	557					
7			187		123								—
9	144		175		126								1.1
	144	144	178	177	123	125							
10	110		176		125								1.5
	109	110	172	174	125	125							
12	108		171		121								1.1
	110	109	169	170	121	121							
13	203		174		122								0.9
	204	204	174	174	120	121							
17	117		173		123		515						2.7
	124	124	175	176	125	124	524	520					
17	132		179		125		522						—
					122								
	117 ^a		174 ^a		119 ^a		546 ^a						

^a Calculated at infinite dilution.

(Continued)

Table 1. (Continued)

Coll. No.	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		σ
	M.W. 202.7		M.W. 172.2		M.W. 122.1		M.W. 536.0		M.W. 123.1		M.W. 891.5		
	Av.		Av.		Av.		Av.		Av.		Av.		
19	206		146		214		521						—
21	140		172						125		854		
	136	138	172	172					122	124	855	855	2.0
25	123		171		195								
					205								
	119	121	173	172	197	199							4.8
28	140		164		117		537						
	151	146	169	167	117	117	522	530					5.0
35	114		167						123				
	116	115	173	170					124	124	791	791	2.6
36	105		172						123		890		
	107	106	170	171					123	123	866	888	1.2
40	151		166						129		839		
	164	158	164	165					137	133	737	788	6.3
41	203		173						124		899		
	201	202	170	172					122	123	876	888	1.7
48	203		164						123				
	202	203	171	168					124	124	875	875	2.9
54	158		170						126		728		
	150	154	164	167					120	123	741	736	4.7
55	97		166						121				
	105	104	170	168					125	123	913	913	4.0
	110												
59	140		175						139		822		
	138	139	172	173					138	139	852	836	1.5
\bar{X}			172.3		123.4		531		125.9		845		(4.1)
σ			10.0		4.6		18.3		5.5		54.4		
Coeff. of var. (%)			5.8		3.7		3.4		4.4		6.4		

used solvents, only seven values were reported. This sample was chosen so that at least one compound in the 500 molecular weight range would be included in the study; unfortunately, the Associate Referee was not aware of the solubility problem when the sample was sent to the collaborators. Although the range of molecular weights obtained seems large, the coefficient of variation, 3.4, is in line with that obtained for the other samples.

Because of the carboxyl group, molecular weight values obtained for nicotinic acid (sample 3), like benzoic acid, were too high unless a polar solvent was used. Eight of the ten values were within one molecular weight unit of the true value. Two high values caused the average to be high by 2.8 units

and the standard deviation and coefficient of variation to be 5.5 and 4.4, respectively. All but one of the values for the highest molecular weight sample, tristearin, were lower than the theoretical molecular weight, and the precision both within and between laboratories was not as good as for the other samples even on a percentage basis. This greater variability is probably due to the lower signal obtained because of the lower concentration of the tristearin solutions. Even with the limited amount of sample sent to the collaborators and the lower concentrations used, the coefficient of variation, 6.4, was only slightly higher than that obtained for the other samples.

Within-laboratory standard deviations ranged from 0.9 to 21.9 but the median

Table 2. Solvent and reference standard used for each analysis reported in Table 1

Coll. No.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
0	water NaCl	acetone benzil	acetone benzil	CHCl ₃ benzil	EtOH-H ₂ O malic acid	CHCl ₃ benzil
0		acetone o-anisidine	acetone anisic acid			
1	water dextrose	ethyl acetate benzil	ethyl acetate benzil	CHCl ₃ benzil		
2	water sucrose	acetone benzil	acetone benzil			
3	benzil	benzil	benzil			
6	water sucrose	water sucrose	CCl ₄ dimethyl terephthalate	CCl ₄ dimethyl terephthalate		
7		methanol benzil	methanol benzil			
9	acetanilide	acetanilide	acetanilide			
10	methanol benzil	acetone benzil	acetone benzil			
12	water sucrose	ethanol o-dinitrobenzene	ethanol			
13	water dextrose	M iso-B K benzoic acid	CCl ₄ benzoic acid			
17	methanol benzil	acetone benzil	acetone benzil	toluene benzil		
19	EtOH (95%) NH ₂ OH·HCl	EtOH (95%) biphenyl	CHCl ₃ biphenyl	CHCl ₃ biphenyl		
21	triphenyl	methane			methane	methane
25	methanol benzil	methanol benzil	benzene benzil			
28	ethanol acetanilide	ethanol acetanilide	ethanol benzoic acid	CHCl ₃ triphenyl PO ₄		
35	methanol benzil	methanol benzil			methanol benzil	CHCl ₃ biphenyl
36	methanol benzil	methanol benzil			methanol benzil	CHCl ₃ ^c
40	ethanol benzil	acetone benzil			acetone benzil	benzene benzil
41	EtOH (95%) NaCl	acetone ^b			EtOH:H ₂ O (99:1) acetanilide	CHCl ₃ ^b
48	water s-benzil	water sugar			water sugar	MEK trimyristin
54	benzil	benzil			benzil	benzil
55	water ^a	acetone azobenzene			water ^a	benzene azobenzene
59	alcohol	acetone			alcohol	CHCl ₃

^a Methyl α-D-glucopyranoside.^b 1,2,4,5-Tetrachlorobenzene.^c Pentaerythrityl tetrabenzene sulfonate.

Table 3. Technical details of method and apparatus used by collaborators^a

Coll. No.	Soln. Equilib. Time	No. Drops	Read in (Min.)	Ref. Therm. Rinsed	Sens. Therm. Rinsed	Thermistor Coating	Volts	Calc. From ΔR or Calib. Curve	Approx. Conc'n, %	Concn, w/w or w/v
0	15	5	4	no	yes	glass	4.2	ΔR	1.2	w/w
1	20	10-15	3	no	no	bare	—	ΔR	0.3	w/v
2	15	7	2	yes	no	glass	0.5	ΔR	0.7	w/w
3	25	5	5	yes	yes	bare	0.5	CC	0.4-1.0	w/v
6	4	3	4	yes	yes	bare	1.5	CC	0.4-1.2	w/v
7	15	6-8	2	no	yes	bare	1.5	CC	0.1	w/v
9	30	7	2-5	no	yes	glass	0.5	ΔR	3.0	w/v
10	60	6	3-5	no	yes	glass	0.5	CC	0.2	w/w
12	15	8	5	yes	no	glass	—	CC	0.5	w/w
13	2-3	4-5	2	yes	yes	glass	1.5	CC	0.5	w/w
17	30	6-8	3-5	no	no	glass	0.5	ΔR	0.5-1.0	w/v
19	15	10	2	no	no	plastic	0.5	CC	0.3-1.0	w/w
21	5	4-5	2	yes	no	glass	0.5	ΔR	0.2	w/v
25	30	8	10	yes	yes	glass	—	CC	1.5	w/w
28	40	6	4-8	no	no	bare	0.5	CC	0.4-1.2	w/v
35	30	6-8	2-10	no	no	plastic	0.5	CC	0.25	w/v
36	15	5	3-6	yes	yes	bare	1.4	ΔR	1.3	w/w
40	3-5	4-6	2	no	no	glass	1.4	ΔR	0.2-1.0	w/v
41	3	5-15	Max ΔR	no	yes	glass	3	ΔR	1.5	w/w
48	30	5	2	no	no	glass	0.5	ΔR	0.5-1.0	w/w
54	15-20	6	3-6	yes	yes	bare	1.35	ΔR	0.2-0.7	w/w
55	30	8-10	2	yes	yes	plastic	—	CC	1.6	w/v
59	4	10	2	no	yes	glass	1.4	CC	0.5	w/v

^a Only those details which differed considerably are shown. Only collaborators 0, 12 and 41 used homemade apparatus.

value was only 2.6. High σ values seemed to be associated with the use of bare thermistors. The average σ for those collaborators using bare thermistors was 8, compared to 2.6 for those using coated thermistors.

Conclusions and Recommendation

These general statements seem warranted from the results of this study:

1. If a sample contains an atom or group which may ionize, check this point and choose the reference standard accordingly.
2. To avoid hydrogen bonding, use as polar a solvent as the sample will dissolve in.
3. Use either glass or plastic-coated thermistors rather than bare thermistors.

It is recommended that this study be continued.

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